Human IL-6 ELISA Kit

Catalog Number：RK00004

This ELISA kit used for quantitative determination of human interleukin 6 (IL-6) concentrations in cell culture supernates, serum and plasma. For research use only, and it's highly recommended to read throughly of this manual before using the product.

Manufactured by

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Contents

Introduction

Interleukin 6 (IL-6) is a pleiotropic, α-helical, 22-28 kDa phosphorylated and variably glycosylated cytokine that plays important roles in the acute phase reaction, inflammation, hematopoiesis, bone metabolism, and cancer progression (1-5). Mature human IL-6 is 183 amino acids (aa) in length and shares 39% aa sequence identity with mouse and rat IL-6 (6). Alternative splicing generates several isoforms with internal deletions, some of which exhibit antagonistic properties (7-10). Cells known to express IL-6 include CD8 + T cells, fibroblasts, synoviocytes, adipocytes, osteoblasts, megakaryocytes, endothelial cells (under the influence of endothelins), sympathetic neurons, cerebral cortex neurons, adrenal medulla chromaffin cells, retinal pigment cells, mast cells, keratinocytes, Langerhans cells, fetal and adult astrocytes, neutrophils, monocytes, eosinophils, colonic epithelial cells, B1 B cells and pancreatic islet beta cells (2, 11-33). IL-6 production is generally correlated with cell activation and is normally kept in control by glucocorticoids, catecholamines, and secondary sex steroids (2). Normal human circulating IL-6 is in the 1 pg/mL range, with slight elevations during the menstrual cycle, modest elevations in certain cancers, and large elevations after surgery (34-38).

IL-6 induces signaling through a cell surface heterodimeric receptor complex composed of a ligand binding subunit (IL-6 R alpha) and a signal transducing subunit (gp130). IL-6 binds to IL-6 Rα, triggering IL-6 Rα association with gp130 and gp130 dimerization (39). gp130 is also a component of the receptors for CLC, CNTF, CT-1, IL-11, IL-27, LIF, and OSM (40). Soluble forms of IL-6 Rα are generated by both alternative splicing and proteolytic cleavage (5). In a mechanism known as trans-signaling, complexes of soluble IL-6 and IL-6 Rα elicit responses from gp130- expressing cells that lack cell surface IL-6 Rα (5). Trans-signaling enables a

wider range of cell types to respond to IL-6, as the expression of gp130 is ubiquitous, while that of IL-6 Rα is predominantly restricted to hepatocytes, monocytes, and resting lymphocytes (2, 5). Soluble splice forms of gp130 block trans-signaling from IL-6/IL-6 Rα but not from other cytokines that use gp130 as a co-receptor (5, 41).

IL-6, along with TNF-α and IL-1, drives the acute inflammatory response. IL-6 is almost solely responsible for fever and the acute phase response in the liver, and it is important in the transition from acute inflammation to either acquired immunity or chronic inflammatory disease (1-5). When dysregulated, it contributes to chronic inflammation in conditions such as obesity, insulin resistance, inflammatory bowel disease, arthritis, and sepsis (2, 5). IL-6 modulates bone resorption and is a major effector of inflammatory joint destruction in rheumatoid arthritis through its promotion of Th17 cell development and activity (1). It contributes to atherosclerotic plaque development and destabilization as well as the development of inflammation-associated carcinogenesis (1, 2). IL-6 can also function as an anti-inflammatory molecule, as in skeletal muscle where it is secreted in response to exercise (2). In addition, it enhances hematopoietic stem cell proliferation and the differentiation of memory B cells and plasma cells (42).

Principle Of The Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for IL-6 is added to the wells and binds to the combination of capture antibody-IL-6 in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps, a substrate is added. A colored product TMB is formed in proportion to the amount of IL-6 present in the sample. The reaction is terminated by addition of acid and absorbance is measured. A standard curve is prepared from seven IL-6 standard dilutions and IL-6 sample concentration determined.

Materials Provided

Part	Size (96T)	Cat NO.	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Antibody Coated Plate	**8×12**	**RM00044**	Return unused wells to the foil pouch containing the desiccant pack and store at ≤ -20 °C.Reseal along entire edge of zip-seal.
Standard Lyophilized	**2**	**RM00041**	Aliquot and store at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Concentrated Biotin Conjugate Antibody (100×)	**1 ×120ul**	**RM00042**	May be stored for up to 6 month at 2-8 °C.*
Streptavidin-HRP Concentrated (100×)	**1 ×120ul**	**RM00043**	
Standard/Sample Diluent (R1)	**1 ×20mL**	**RM00023**	May be stored for up to 6 month at 2-8 °C.*
Biotin-Conjugate Antibody Diluent (R2)	**1 ×12mL**	**RM00024**	
Streptavidin-HRP Diluent(R3)	**1 ×12mL**	**RM00025**	
Wash Buffer(20x)	**1 × 30mL**	**RM00026**	
TMB Substrate	**1 ×12 mL**	**RM00027**	
Stop Solution	**1 ×6 mL**	**RM00028**	
Plate Sealers	**4 strips**		
Specification	**1**		

Sample Collection And Storage

1. **Cell Culture Supernates:**

   Centrifuge 1000x g for 10 min and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.If cell culture supernate samples require larger dilutions, perform an intermediate dilution with culture media and the final dilution with the Standard/Sample Diluent(R1).

2. **Serum:**

   Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000x g, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.

3. **Plasma**

   Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze / thaw cycles.

4. **Avoid hemolytic and hyperlipidemia sample for Serum and Plasma.**

5. **Dilution:**

   Dilute samples at the appropriate multiple (recommend to do pre-test to determine the dilution factor).

Precautions

1. **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
2. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
3. Variations in sample collection, processing, and storage may cause sample value differences.
4. Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.
5. Stop Solution contains strong acid. Wear eye, hand, and face protection.
6. Apart from the standard of kits, other components should not be refrigerated.
7. Please perform simple centrifugation to collect the liquid before use.
8. Do not mix or substitute reagents with those from other lots or other sources.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
10. Mix the sample and all components in the kits adequately, and use clean plastic container to prepare all of the diluent.
11. Both the sample and standard should be assayed in duplicate, and the sequence of the regents should be added consistently.
12. Reuse of dissolved standard is not recommended.
13. The kit should not be used beyond the expiration date on the kit label.
14. The kit should be away from light when it is stored or incubated.
15. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
16. To avoid cross contamination, please use disposable pipette tips.

17. Please prepare all the kit components according to the Specification. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
18. The 48T kit is also suitable for the specification.

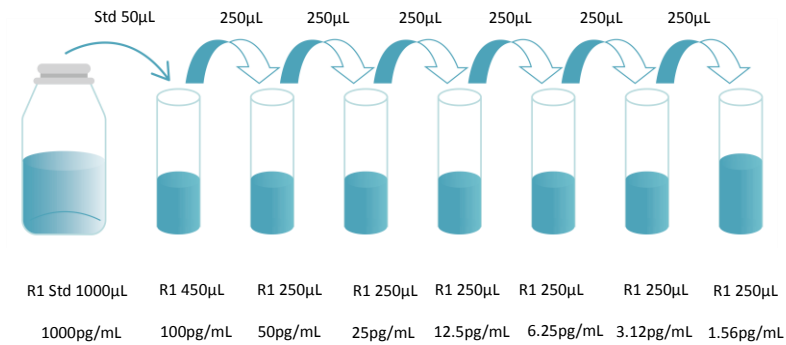
Experiment Materials

1. Microplate reader(measuring absorbance at 450 nm, with the correction wavelength set at 570 nm or 630 nm).
2. Pipettes and pipette tips：0.5-10, 2-20, 20-200, 200-1000 μL.
3. Microplate washer, Squirt bottle.
4. Micro-oscillator.
5. Deionized or double distilled water, graduated cylinder.
6. Polypropylene Test tubes for dilution.
7. Incubator.

Reagent Preparation

1. **Bring all reagents to room temperature before use. If crystals have formed in the concentrate, Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.**

2. **Standard:** Add Standard/Sample Diluent(R1) 1.0mL into freeze-dried standard, sit for a minimum of 15 minutes with gentle agitation prior to making dilutions (1000pg/mL), Prepare EP tubes containing Standard/Sample Diluent(R1), and produce a dilution series according to the picture shown below (recommended concentration for standard curve: 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0 pg/mL). Redissolved standard solution (1000 pg/mL), aliquot and store at -20°C— -70°C.



3. **Concentrated Biotin Conjugate Antibody (100x)** : Dilute 1:100 with the Biotin-Conjugate Antibody Diluent (R2) before use, and the diluted solution should be used within 30 min.

Dilution Method

Strip	Concentrated Biotin-Conjugate antibody (100x)	Biotin-Conjugate Antibody Diluent (R2)
2	20ul	1980ul
4	40ul	3960ul
6	60ul	5940ul
8	80ul	7920ul
10	100ul	9900ul
12	120ul	11880ul

4. **Streptavidin-HRP Concentrated (100x):** Dilute 1:100 with the Streptavidin-HRP Diluent(R3) before use, and the diluted solution should be used within 30 min.

Dilution Method

Strip	Concentrated Streptavidin-HRP (100x)	Streptavidin-HRP Diluent(R3)
2	20ul	1980ul
4	40ul	3960ul
6	60ul	5940ul
8	80ul	7920ul
10	100ul	9900ul
12	120ul	11880ul

5. **Wash buffer**: Dilute 1:20 with double distilled or deionized water before use.

Wash Method

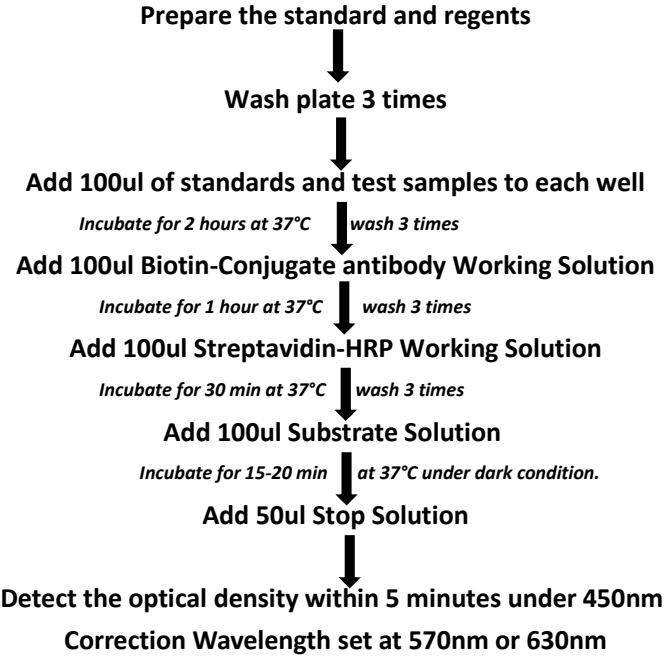
Aspirate each well and wash, repeating the process two times for a total of three washes.Wash by filling each well with **Wash Buffer**(300ul) using a squirt bottle,manifold dispenser,or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash,remove any remaining **Wash Buffer** by aspirating or decanting.Invert the plate and blot it against clean paper towels.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Add wash buffer 300 µL/well, aspirate each well after holding 40 seconds, repeating the process two times for a total of three washes.
3. Add 100 µL Standard/sample Diluent (R1) in blank well.
4. Add 100 µL different concentration of standard and sample in other wells, cover with the adhesive strip provided. Incubate for 2 hours at 37°C.
5. Repeat the aspiration/wash as in step 2.
6. Prepare the Concentrated Biotin Conjugate Antibody (100X) Working Solution 15 minutes early before use.
7. Add Biotin-Conjugate Antibody Diluent(R2) in blank well and Biotin-Conjugate antibody Working Solution in other wells (100µL/well), cover with new adhesive strip provided. Incubate for 1 hour at 37°C.
8. Prepare the Streptavidin-HRP Concentrated (100X) Working Solution 15minutes early before use.
9. Repeat the aspiration/wash as in step 2.
10. Add Streptavidin-HRP Diluent(R3) in blank well and add Streptavidin-HRP Working Solution in other wells (100 µL/well), cover with new adhesive strip provided. Incubate for 30 minutes at 37°C.
11. Warm-up the Microplate reader.
12. Repeat the aspiration/wash as in step 2.
13. Add TMB Substrate (100µL/well). Incubate for 15-20 minutes at 37°C .Protect from light.
14. Add Stop Solution (50µL/well), determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength

correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

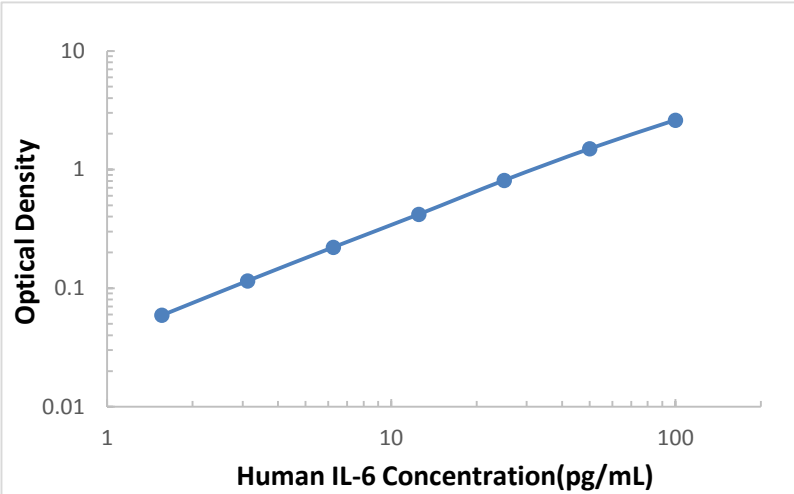
Assay Procedure Summary



Calculation Of Results

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).
2. Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the IL-6 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted,the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data



The standard curves are provided for demonstration only. A standard curve should be generated for each set of IL-6 assayed.

Sensitivity

The minimum detectable dose (MDD) of IL-6 is typically less than 0.7 pg/mL. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay recognizes both recombinant and natural human IL-6. The factors listed below were prepared at 50ng/ml and assayed for cross-reactivity. No significant cross-reactivity was observed with the following:

Recombinant human	Recombinant mouse	Recombinant rat
CNTF	GM-CSF	CNTF
G-CSF	IL-2	
GM-CSF	IL-3	
sgp130	IL-4	
IL-1α	IL-5	
IL-1β	IL-6	
IL-2	IL-7	
IL-3	IL-11	
IL-4	IL-12	
IL-6 Rα/sgp130		
IL-7		
IL-8		
IL-11		
IL-12		
LIF		
LIF R		
OSM		
TNF-α		
TNF-β		

Precision

Intra-plate Precision

Three samples of known concentration were tested 20 times on one plate to evaluate the Intra-plate precision.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	114	576	1220
Standard Deviation (SD)	5.1	28.8	57.3
Variable Coefficient CV (%)	4.5	5.0	4.7

Inter-plate Precision

Three samples of known concentration were tested 20 times separate assays to evaluate the Inter-plate precision.Assay were using two lots of components.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	215	894	1763
Standard Deviation (SD)	14	63.5	132.2
Variable Coefficient CV (%)	6.5	7.1	7.5

Recovery

Spike 3 different concentration of human IL-6 into healthy human serum and plasma, calculate the recovery.

Sample Form	Average Recover (%)	Range (%)
Serum	98	88-108
Plasma	104	91-116

Linearity

Spike high concentration of human IL-6 into 4 healthy human serum, dilute in the range of standard curve kinetics and evaluate the linearity.

Dilution	Average Value (%)	Range (%)
1:2	97	85-108
1:4	102	92-111
1:8	97	89-105
1:16	99	94-103

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